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Health and Environmental Effects Profile for Azobenzene

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR AZOBENZENE

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16. ABSTRACT

The Health and Environmental Effects Profile for azobenzene was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates have been presented provided sufficient data are available. Azobenzene has been evaluated as a carcinogen. The human carcinogen potency factor (q_1^*) for azobenzene is 1.08×10^{-1} $(mg/kg/day)^{-1}$ for oral exposure.

The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified by CERCLA based on chronic toxicity. The RQ value for azobenzene is 100.

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PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste and Emergency Response by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA), as well as to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched." The literature search material is current through November, 1984.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Acceptable Daily Intakes (ADIs) for chronic exposures. An ADI is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, ADIs are not estimated in this document series. Instead, a carcinogenic potency factor of q_1^* (U.S. EPA, 1980) is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under CERCLA. These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity and acute mammalian toxicity).

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA and CERCLA dockets.

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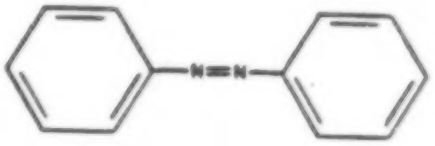
LIST OF ABBREVIATIONS

BCF	Bioconcentration factor
CAS	Chemical Abstracts Service
CS	Composite score
DMBA	7, 12-dimethylbenz(a)anthracene
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
K_{oc}	Soil sorption coefficient
K_{ow}	Octanol/water partition coefficient
MED	Minimum effective dose
MTD	Maximum tolerated dose
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
ppm	Parts per million
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value
TLm	Median tolerance limits
TWA	Time-weighted average
UDS	Unscheduled DNA synthesis
UV	Ultraviolet

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

Azobenzene is known by the following synonyms: azobenzeen (Dutch); azobenzide; azobenzol; azobisbenzene; azodibenzene; azodibenzeneazofume; azofume; benzene,azobis; benzene,azodi; 1,2-diphenyldiazene; benzeneazobenzene; diphenyldiimide; and nitrogen benzide. Current chemical abstract usage is diazene, diphenyl. Both cis and trans-isomers can exist, with the trans-isomer predominating. Cis-trans interconversions take place both thermally and photochemically (Griffiths, 1972). The structure, empirical formula and molecular weight of azobenzene are given below:



Empirical formula: $C_{12}H_{10}N_2$

Molecular weight: 182.23

The CAS Registry number for this compound is 103-33-3.

1.2. PHYSICAL AND CHEMICAL PROPERTIES

Azobenzene occurs as yellow or orange crystals (Hawley, 1977), orange-red leaflets (Windholz, 1976) or plates (Weast, 1972). Selected physical properties are listed below (Windholz, 1976; Hawley, 1977; Weast, 1972; unless otherwise noted):

Melting point:	68°C	
Boiling point:	293°C	
Specific gravity:	1.203_4^{20}	
Vapor pressure:	1 mm Hg at 103.5°C	Toxicology Data Bank, 1979
Index of refraction:	1.6266 at 78°C	

Wavelength of UV absorption maxima (nm):	433, 314, 228	Sadtler, 1966
Corresponding absorption coefficient (ℓ/mol-cm):	729, 18,200, 13,400	
Water solubility:		
at 25°C	6.7 mg/ℓ	Takagishi et al., 1969
at 25°C	302 mg/ℓ	Briggs, 1981
	4.6 mg/ℓ	Lyman et al., 1982
Nonaqueous solvent solubility:	soluble in glacial acetic acid, alcohol, ether, benzene, pyridine, methanol, chloroform	
Log K_{ow}:	3.82	Hansch and Leo, 1981 Briggs, 1981
Log acid dissociation constant:	-2.95	Hoefnagel et al., 1969
Log K_{oc}: [calculated on a weight (µg/g) basis]	3.13 (4 Balcombe silt loams, pH 6-7.5, 20°C, 1.09-4.25% organic matter)	Briggs, 1981
	3.31 (sterilized) 3.44 (unsterilized) (Hagerstown clay loam; pH 6.6; 20°C; 34% clay, 42% silt, 2.5% organic matter)	Pillai et al., 1982
	3.73 (sterilized) 3.81 (unsterilized) (Palouse silt loam; pH 5.1; 20°C; 25% clay, 58% silt, 3.4% organic matter)	Pillai et al., 1982

1.3. PRODUCTION DATA

Azobenzene is usually prepared by the controlled reduction of nitrobenzene with iron in the presence of caustic soda (Sandridge and Staley, 1978). A mixture of nitrobenzene and fine iron borings are stirred and heated to ~90°C and a 60% caustic soda solution is added gradually with the temperature maintained at 100-120°C. A paste of iron and caustic soda converts the

azoxybenzene intermediate into azobenzene. Azobenzene can also be obtained by boiling nitrobenzene with an alcoholic solution of sodium hydroxide followed by distillation of the azoxybenzene intermediate in the presence of finely divided iron, and from reduction of nitrobenzene with zinc dust and alkali followed by oxidation of the intermediate hydrazobenzene with ferric chloride. Oxidation of aniline by air or oxygen at room temperature yields azobenzene. It also can be obtained by refluxing an aniline solution with manganese dioxide (Northcott, 1978). Electrolytic reduction of nitrobenzene (Windholz, 1976), as well as reduction with sodium stannite, have also been reported as a method of synthesis of azobenzene (Hawley, 1977).

Radding et al. (1977) estimated that <1000 pounds of azobenzene were produced in the United States in 1974. The TSCA production inventory listed only Bofors Lakeway Inc. in Muskegon, MI as a commercial manufacturer of azobenzene in the United States with a level of production between 100,000 and 1 million pounds per year (U.S. EPA, 1985). Table 1-1 lists some of the distributors of azobenzene in the United States.

USITC (1984) reported azobenzene (technical grade) imports of 84,028 pounds in 1983.

1.4. USE DATA

Azobenzene has been used as a photosensitive component of single layer configuration deformation media for temporary or permanent image recording. For this use, azobenzene is dissolved in an inert thermoplastic polymer such as polystyrene (Aftergut, 1979).

Reportedly, azobenzene previously was used in the manufacture of rubber accelerators, dyes and as a fumigant (Hawley, 1977). The NCI (1979) reported that although azobenzene may occur as a by-product during the manufacture of benzidine, a precursor of azo dyes, azobenzene itself is not used as a dyestuff. Azobenzene was also used as an acaricide (U.S. EPA, 1981).

TABLE 1-1

Azobenzene Distributors in the United States*

Distributor	Location
Analabs, Inc.	North Haven, CT
J.T. Baker Chemical Co.	Phillipsburg, NJ
Chemical Procurement Labs, Inc.	College Point, NY
Chem Service, Inc.	West Chester, PA
Dynamitt Nobel of America, Inc.	Northvale, NJ
EM Laboratories, Inc.	Elmsford, NY
Eastern Chemical	Hauppauge, NY
Eastman Organic Chemicals	Rochester, NY
Fisher Scientific Co.	Pittsburgh, PA
Atomergic Chemetals Corp.	Plainview, NY
Haven Chemical	Philadelphia, PA
ICN/K&K	Plainview, NY
Lachat Chemicals, Inc.	Meguon, WI
MC&B Manufacturing Chemists	Cincinnati, OH
Mallinckrodt, Inc.	St. Louis, MO
Mide Chemical Corp.	Bohemia, NY
Tridom Chemical, Inc.	Hauppauge, NY
VWR Scientific	San Francisco, CA

*Source: Baker et al., 1978

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

2.1. AIR

Azobenzene has a low vapor pressure (i.e., 1 mm Hg at 103.5°C) (Toxicology Data Bank, 1979) and would therefore not be expected to be very volatile. Radding et al. (1977) reported that since azo groups are strong chromophores that efficiently absorb visible and near UV solar radiation, azobenzene may undergo a variety of photochemical transformations. While no data regarding the gas phase photodegradation of azobenzene were located in the available literature cited in the Appendix, the information reported regarding solution phase photodegradation (Section 2.2.) suggests that any azobenzene dispersed in the atmosphere would be expected to be subjected to photodegradation. Furthermore, Radding et al. (1977) also reported that any azo dye, which may be dispersed in the atmosphere, is likely to be oxidized by OH radicals at rates comparable with those for other aromatics (i.e., half-lives of $\leq$8 hours). Therefore, azobenzene is also expected to react significantly with OH radicals in the atmosphere. A report by Kerzhner et al. (1980) concerning the oxidation of azobenzene with ozone in aqueous solution (Section 2.2.) suggests that this reaction may also be possible with atmospheric azobenzene.

In view of the above information, azobenzene is not expected to be present in any significant amounts in the atmosphere. The residence time of atmospheric azobenzene is anticipated to be short because of its photodegradation and oxidation by OH radicals and ozone in the atmosphere.

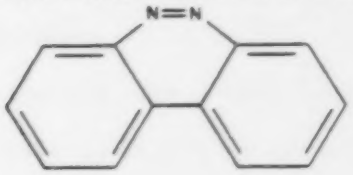
Data concerning atmospheric transport of azobenzene could not be located in the available literature as cited in the Appendix.

2.2. WATER

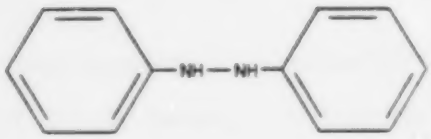
Conflicting data concerning the water solubility of azobenzene occur in the literature. Takagishi et al. (1969) reported a solubility of ~6.7 mg/ℓ at 25°C; whereas, Briggs (1981) reported a solubility of 302 mg/ℓ at 25°C. The lower level of solubility of azobenzene seems to be supported by the estimated solubility data (4.6 ppm) based on the K_{ow} and the regression provided by Lyman et al. (1982).

The photochemistry of azobenzene has been studied extensively. Ali and Al-Saigh (1980) reported the photodecomposition of azobenzene in cyclohexane and ether solutions contained in a Hanovia-type photochemical reactor and irradiated with a filtered medium pressure mercury vapor arc lamp that emitted radiations at 254, 265, 297, 313, 365, 404 and 436 nm. The UV spectrum of azobenzene exhibits absorptions at 228, 314, 433 nm (Sadtler, 1966; see Section 1.2.). The major products formed in cyclohexane solution are N-cyclohexylaniline, phenylazocyclohexane and 1-phenyl-2-cyclohexylhydrazine (Ali and Al-Saigh, 1980). The major products formed in diethylether are N-polymethylenephenylhydrazine, phenylhydrazine cyclohexane and phenylazo-N-polymethylene. Initial decomposition products upon irradiation were identified as phenylazo, phenylazomethylene and aniline radicals. About 100 hours of irradiation time were required for 10^{-3} M azobenzene solutions in cyclohexane to achieve complete decomposition. Griffiths (1972) reviewed the photochemistry of azobenzene and its derivatives. Both photocyclization and photoreduction may occur with azobenzene. The photocyclization of azobenzene in 98% sulfuric acid produced benzo[c]cinnoline; however, quantum yield studies indicated that photocyclization was an inefficient process (quantum yield of ~0.015). The photoreduction of azobenzene produced

hydrazobenzene, which was subsequently converted to benzidine. The efficiency of this reaction was found to be solvent-dependent. The structures for benzo-[c]-cinnoline and hydrazobenzene are shown below:



benzo - [c] - cinnoline



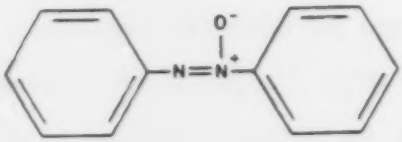
hydrazobenzene

Meir (1971) reported on the photochemistry of azo dyes and noted that both oxidation and reduction of azo dyes occur slowly. Radding et al. (1977) concluded that in aquatic systems where natural organics are present at concentrations of 5-10 ppm along with oxygen, photoreduction would probably be very slow (if it occurred at all), except in oxygen-poor water.

The data above suggest that azobenzene in aqueous solution can be slowly degraded by sunlight, especially near the surface.

Data concerning the hydrolysis of azobenzene could not be located in the available literature as cited in the Appendix.

The oxidation of azobenzene with ozone in dilute aqueous solutions predominantly produced azoxybenzene (Kerzhner et al., 1980). The reaction was first order with respect to O_3 with a rate constant of 1.01 ℓ/mol minute at 25°C. The structure for azoxybenzene is shown below.



Data regarding the biodegradation of azobenzene in aqueous media could not be located in the available literature as cited in the Appendix. Results of biodegradation studies in soils, however, suggest that azobenzene may be resistent to biodegradation in aqueous systems (Bordeleau and Bartha,

1972; Pillai et al., 1982) (Section 2.3.). Malaney (1960) reported that azobenzene is resistant to degradation by aniline-acclimated sludge. Therefore, the biodegradation of azobenzene in aquatic systems is probably not significant.

The U.S. EPA (1981) suggested that since azobenzene is nonvolatile and slightly polar, it is expected to exhibit little tendency to transfer from water to the atmosphere. Furthermore, its lipophilicity may lead to significant absorption by sediments with high organic content. A potential for bioaccumulation was suggested because of the relatively high log K_{ow} value of 3.82 (U.S. EPA, 1981). An estimated BCF of 470 was calculated based on the log K_{ow} using the estimation methods of Lyman et al. (1982). Based on the estimated BCF value of 470, azobenzene would not be expected to strongly bioconcentrate in aquatic organisms.

Zoeteman et al. (1980) reported a half-life of 1.4 days for azobenzene in water of the Rhine River in the Netherlands. The authors categorized azobenzene as slightly persistent.

2.3. SOIL

Azobenzene has been reported to be one of the products of biodegradation of aniline both in soil and by partially purified enzymes of the soil fungus, Geotichum candidum (Bordeleau and Bartha, 1972). The fact that no further degradation of azobenzene was reported suggests that azobenzene was resistant to biodegradation. Pillai et al. (1982) reported a study of the soil-catalyzed oxidation of aniline in which not only azobenzene, but also azoxybenzene, phenazine, formanilide and acetanilide were formed; however, further transformation of the azobenzene was not investigated in this study.

The above information as well as the Malaney (1960) study, which reported that azobenzene was resistant to biodegradation by aniline activated sludge (see Section 2.2.), suggests that azobenzene may be resistant to biodegradation in soils.

Data concerning the solution phase photodegradation of azobenzene (see Section 2.2.) suggest that significant photodegradation of azobenzene may not occur in soil.

Data concerning the hydrolysis of azobenzene in soil could not be located in the available literature as cited in the Appendix.

Briggs (1981) reported an average K_{oc} of 1349 in a study of four soil samples of Balcombe silt loams from arable land with organic matter contents of 1.09-4.25%, pH 6-7.5 and a temperature of 20°C. Pillai et al. (1982) reported K_{oc} values of 2030 (sterilized soil) to 2750 (unsterilized soil) for azobenzene in Hagerstown clay loam (34% clay, 42% silt, 2.5% organic matter, pH 6.6 and 20°C). Also reported were K_{oc} values of 5390 (sterilized soil) to 6410 (unsterilized soil) for azobenzene in Palouse silt loam (25% clay, 58% silt, 3.4% organic matter, pH 5.1, and 20°C). Therefore, azobenzene would be expected to be substantially sorbed by the soil. It appears that substantial variation in the extent of sorption of azobenzene may occur depending on the organic carbon content of the soil in question.

Data concerning the behavior of azobenzene in groundwater could not be located in the available literature as cited in the Appendix.

3. EXPOSURE

3.1. WATER

The U.S. EPA STORET Data Base reports the analysis of azobenzene in 152 water samples. The mean concentration of azobenzene in the samples was reported to be 5.125 µg/ℓ, with a range of 5.00-12.00 µg/ℓ.

Zoeteman et al. (1980) reported an azobenzene concentration level of 0.3 µg/ℓ for surface water from the Rhine River in the Netherlands. Shackelford and Keith (1976) detected azobenzene in chemical plant effluent, but no details were given. Jungclaus et al. (1978) detected 30 µg/ℓ azobenzene in one sample of wastewater from a specialty chemicals plant, but no azobenzene was found in the receiving river water, sediment or tarballs from the river bottom.

3.2. FOOD

Monitoring data for azobenzene exposure in food could not be located in the available literature as cited in the Appendix.

3.3. INHALATION

Monitoring data for azobenzene exposure in the air could not be located in the available literature as cited in the Appendix.

3.4. DERMAL

Monitoring data for azobenzene exposure on the skin could not be located in the available literature as cited in the Appendix.

4. PHARMACOKINETICS

4.1. ABSORPTION

Bray et al. (1951) reported that azobenzene was not completely absorbed in rabbits. Approximately 30% of a presumably oral dose of 500 mg/kg was found in the feces; the percentage of the dose found in the urine was not reported and biliary excretion was not investigated.

4.2. DISTRIBUTION

Pertinent data regarding the distribution of azobenzene could not be located in the available literature as cited in the Appendix.

4.3. METABOLISM

After treatment of male rats by intraperitoneal injection of 50 mg azobenzene, aniline and a water soluble compound that yields benzidine after acidification were found in the urine (Elson and Warren, 1944). The authors suggested that the water soluble product was hydroazobenzene which was rapidly excreted as a glucuronide or sulphate conjugate. Bray et al. (1951) reported that ~60% of the absorbed azobenzene was excreted by rabbits as the glucuronide, ~20% as the sulphate and ~23% was unchanged. Small amounts of free or conjugated forms of o- and p-hydroxyazobenzene, benzidine, o- and p-acetamidophenol, hydrazobenzene and o- and p-aminophenol, hydrazobenzene and o- and p-aminophenol were also detected in the urine.

Fouts et al. (1957) reported that the azo linkage of absorbed color was reduced in rabbit liver homogenate by microsomal NADPH-dependent reductase.

4.4. EXCRETION

Bray et al. (1951) reported that ~30% of the administered dose of azobenzene to rabbits were found in the feces and that ~60% of the absorbed dose of azobenzene was excreted as the glucuronide, ~20% as the sulphate and ~23% as unchanged compound.

5. EFFECTS

5.1. CARCINOGENICITY

An early bioassay evaluated the carcinogenic potential of 130 industrial chemicals including azobenzene (Innes, 1968; Innes et al., 1969). Results of the chronic feeding and subcutaneous administration studies of azobenzene in mice are summarized in Table 5-1. Eighteen male and 18 female mice from each hybrid strain (B6AKF1 and B6C3F1) were selected for the 18-month study for each route of administration. An equal number of mice served as untreated controls and a group of 36 mice served as vehicle controls for each solvent. MTD values were determined in a preliminary acute toxicity study and were administered by one of the following two routes: a single subcutaneous injection in 0.05 mL of DMSO in the nape of the neck at 28 days of age; in 0.5% gelatin by stomach tube from 7-28 days of age and then mixed in the feed until termination of the experiment. Following necropsy of the mice killed at ~18 months of age, comprehensive gross and histological examinations were performed. As summarized in Table 5-1, tumors occurred primarily in the liver and lung. When azobenzene was given orally for 80 weeks, an excess of hepatomas, when compared with controls (vehicle and untreated control combined), was found only in male B6C3F1 mice (8/18 in the treated compared with 8/73 in the controls). The incidences of tumors at other sites and hepatomas were comparable between treated and control female B6C3F1 mice and treated and control B6AKF1 male and female mice. The results were considered by the authors as "requiring additional evaluation" (Innes, 1968; Innes et al., 1969).

TABLE 5-1

Carcinogenicity Testing in Mice[a]

Exposure Route	Species/ Strain	Sex	Dose	Duration of Treatment (weeks)	Duration of Study (weeks)	Purity of Compound	Vehicle or Physical State	Target Organ	Tumor Type	Tumor Incidence (%)
Oral	mice/B6C3F1	M	0	80	80	NA	gelatin/diet	liver	hepatoma	8/73 (11)
		M	7.6 mg/kg/day[b]	80	80	commercial	gelatin/diet	liver	hepatoma	8/18[c] (44)
		F	0	80	80	NA	gelatin/diet	liver	hepatoma	0/83 (0)
		F	7.6 mg/kg/day[b]	80	80	commercial	gelatin/diet	liver	hepatoma	0/18 (0)
		M	0	80	80	NA	gelatin/diet	lung	pulmonary adenoma	5/73 (7)
		M	7.6 mg/kg/day[b]	80	80	commercial	gelatin/diet	lung	pulmonary adenoma	0/18 (0)
		F	0	80	80	NA	gelatin/diet	lung	pulmonary adenoma	3/83 (4)
		F	7.6 mg/kg/day[b]	80	80	commercial	gelatin/diet	lung	pulmonary adenoma	1/18 (6)
		M	0	80	80	NA	gelatin/diet	reticulum cell	sarcoma	5/73 (7)
		M	7.6 mg/kg/day[b]	80	80	commercial	gelatin/diet	reticulum cell	sarcoma	0/18 (0)
		F	0	80	80	NA	gelatin/diet	reticulum cell	sarcoma	4/83 (5)
		F	7.6 mg/kg/day[b]	80	80	commercial	gelatin/diet	reticulum cell	sarcoma	0/18 (0)
Oral	mice/B6AKF1	M	0	80	80	NA	gelatin/diet	liver	hepatoma	5/89 (6)
		M	7.6 mg/kg/day[b]	80	80	commercial	gelatin/diet	liver	hepatoma	2/18 (11)
		F	0	80	80	NA	gelatin/diet	liver	hepatoma	1/75 (1)
Oral	mice/B6AKF1	F	7.6 mg/kg/day[b]	80	80	commercial	gelatin/diet	liver	hepatoma	0/18 (0)
		M	0	80	80	NA	gelatin/diet	lung	pulmonary adenoma	9/89 (10)

TABLE 5-1 (cont.)

Exposure Route	Species/ Strain	Sex	Dose	Duration of Treatment (weeks)	Duration of Study (weeks)	Purity of Compound	Vehicle or Physical State	Target Organ	Tumor Type	Tumor Incidence (%)
Oral (cont.)		M	7.6 mg/kg/day[b]	80	80	commercial	gelatin/diet	lung	pulmonary adenoma	1/18 (6)
		F	0	80	80	NA	gelatin/diet	lung	pulmonary adenoma	3/75 (4)
		F	7.6 mg/kg/day[b]	80	80	commercial	gelatin/diet	lung	pulmonary adenoma	2/18 (11)
		M	0	80	80	NA	gelatin/diet	reticulum cell	sarcoma	1/89 (1)
		M	7.6 mg/kg/day[b]	80	80	commercial	gelatin/diet	reticulum cell	sarcoma	0/18 (0)
		F	0	80	80	NA	gelatin/diet	reticulum cell	sarcoma	3/75 (4)
		F	7.6 mg/kg/day[b]	80	80	commercial	gelatin/diet	reticulum cell	sarcoma	1/18 (6)
Subcutaneous[d]	mice/B6C3F1	M	0	80	80	NA	DMSO	liver	hepatoma	1/23 (4)
		M	1000 mg/kg single dose	80	PJ	commercial	DMSO	liver	hepatoma	2/15 (13)
		F	0	80	80	NA	DMSO	liver	hepatoma	0/23 (0)
		F	1000 mg/kg single dose	80	80	commercial	DMSO	liver	hepatoma	0/18 (0)
		M	0	80	80	NA	DMSO	lung	pulmonary adenoma	0/23 (0)
		M	1000 mg/kg single dose	80	80	commercial	DMSO	lung	pulmonary adenoma	0/15 (0)
		F	0	80	80	NA	DMSO	lung	pulmonary adenoma	1/23 (4)
		F	1000 mg/kg single dose	80	80	commercial	DMSO	lung	pulmonary adenoma	1/18 (6)

TABLE 5-1 (cont.)

Exposure Route	Species/ Strain	Sex	Dose	Duration of Treatment (weeks)	Duration of Study (weeks)	Purity of Compound	Vehicle or Physical State	Target Organ	Tumor Type	Tumor Incidence (%)
Subcutaneous[d]	mice/B6C3F1	M	0	80	80	NA	DMSO	reticulum cell	sarcoma	0/23 (0)
		M	1000 mg/kg single dose	80	80	commercial	DMSO	reticulum cell	sarcoma	2/15 (13)
		F	0	80	80	NA	DMSO	reticulum cell	sarcoma	0/23 (0)
		F	1000 mg/kg single dose	80	80	commercial	DMSO	reticulum cell	sarcoma	0/18 (0)
Subcutaneous[d,e,f]	mice/B6AKF1	M	0	80	80	NA	DMSO	liver	hepatoma	0/22 (0)
		M	1000 mg/kg single dose	80	80	commercial	DMSO	liver	hepatoma	1/17 (6)
		F	0	80	80	NA	DMSO	liver	hepatoma	0/24 (0)
		F	1000 mg/kg single dose	80	80	commercial	DMSO	liver	hepatoma	1/17 (6)

QUALITY OF EVIDENCE

Strengths of Study: Two strains and both sexes of mice were treated for a significant portion of their lifespan. Equal numbers of treated and vehicle controls were used. Part of a large well controlled study. The chemical was administered by two routes.

Weaknesses of Study: Small number of mice per group (18). One dosage level used for each exposure route.

Overall Adequacy: Adequate

[a]Source: Innes, 1968; Innes et al., 1969

[b]TWA dose. The mice were treated by gavage at 21.5 mg/kg/day for 14 days followed by 56 ppm in the diet for 78 weeks.

[c]Greater incidence of hepatomas as compared with controls (8/18 versus 8/73).

[d]Results were similar to treated and untreated controls

[e]Pulmonary adenomas and reticulum cell sarcomas were not found in control or dosed animals of the B6AKF1 strain.

[f]An adenoma of the uterus (1/17) was found in a dosed female of the B6AKF1 strain and not in a control. A malignant lymphoma (1/17) was found in a dosed male of the B6AKF1 strain but not in the dosed females or the controls.

NA - Not applicable

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No increased incidences of tumors were observed in the mice treated with azobenzene subcutaneously compared with controls (Innes, 1968; Innes et al., 1969). A group of 50 Sherman rats treated weekly with subcutaneous injections of 30 mg azobenzene in olive oil (total dose, 3.1 g) also failed to develop local or liver tumors (Spitz et al., 1950).

The NCI (1979) bioassay of azobenzene evaluated its carcinogenicity in F344 rats and B6C3F1 mice. Results of the study are summarized in Table 5-2. Groups of 50 male and 50 female F344 rats and groups of 50 male B6C3F1 mice were given azobenzene in the diet at either 200 or 400 ppm for 105-106 weeks. Female B6C3F1 (groups of 50) mice were given azobenzene at 400 or 800 ppm in the diet for 38 weeks. Because of reduced body weights in the females, doses were lowered to 100 and 400 ppm, respectively, and continued for 67 or 68 weeks. Matched controls consisted of 20 untreated rats of each sex and 20 untreated mice of each sex. Dosages for the chronic study were determined from a subchronic feeding study in rats and mice to estimate the MTDs of azobenzene. All animals were observed twice daily throughout the study. In addition, monthly clinical examination and palpation for masses and body weight determinations were performed. Comprehensive gross and histologic examinations were performed on all animals that died during the study or were killed at the end of the study, except when precluded by cannibalism or autolysis. Peripheral blood smears were also made for all animals.

Dose-related decreased mean body weights of treated rats and mice of both sexes compared with control were observed throughout the study. Dose-related increased mortality was observed in male rats and female mice, but not in male mice or female rats. Survival for all groups regardless of treatment, species or sex was $\geq$70% at week 90 of the study.

TABLE 5-2

Carcinogenicity Testing in Rats and Mice[a]

Agent: Azobenzene

Exposure Route	Species/ Strain	Sex	Dose (ppm)	Duration of Treatment (weeks)	Duration of Study (weeks)	Purity of Compound (%)	Vehicle or Physical State	Target Organ	Tumor Type	Tumor Incidence (p value)[b]
Oral	rat/F344	F	0	105-106	105-106	NA	diet	spleen	fibrosarcoma	0/20 (p=0.012[c])
		F	200	105-106	105-106	99.5	diet	spleen	fibrosarcoma	1/50
		F	400	105-106	105-106	99.5	diet	spleen	fibrosarcoma	7/50
		M	0	105-106	105-106	NA	diet	spleen	fibrosarcoma	0/20 (p=0.020[c])
		M	200	105-106	105-106	99.5	diet	spleen	fibrosarcoma	2/49
		M	400	105-106	105-106	99.5	diet	spleen	fibrosarcoma	7/49
		F	0	105-106	105-106	NA	diet	spleen	osteosarcoma	0/20 (p=0.041[c])
		F	200	105-106	105-106	99.5	diet	spleen	osteosarcoma	1/50
		F	400	105-106	105-106	99.5	diet	spleen	osteosarcoma	5/10
		M	0	105-106	105-106	NA	diet	spleen	osteosarcoma	0/20
		M	200	105-106	105-106	99.5	diet	spleen	osteosarcoma	1/49
		M	400	105-106	105-106	99.5	diet	spleen	osteosarcoma	1/49
		F	0	105-106	105-106	NA	diet	spleen	hemanglosarcoma	0/20
		F	200	105-106	105-106	99.5	diet	spleen	hemanglosarcoma	1/50
		F	400	105-106	105-106	99.5	diet	spleen	hemanglosarcoma	4/50
		M	0	105-106	105-106	NA	diet	spleen	hemanglosarcoma	0/20
		M	200	105-106	105-106	99.5	diet	spleen	hemanglosarcoma	1/49
		M	400	105-106	105-106	99.5	diet	spleen	hemanglosarcoma	4/50
		F	0	105-106	105-106	NA	diet	abdominal cavity	sarcoma	0/20 (p<0.001[c])

TABLE 5-2 (cont.)

Exposure Route	Species/ Strain	Sex	Dose (ppm)	Duration of Treatment (weeks)	Duration of Study (weeks)	Purity of Compound (%)	Vehicle or Physical State	Target Organ	Tumor Type	Tumor Incidence (p value)[b]
Oral	rat/F344	F	200	105-106	105-106	99.5	diet	abdominal cavity	sarcoma	5/50
		F	400	105-106	105-106	99.5	diet	abdominal cavity	sarcoma	21/50 (p<0.001[d])
		M	0	105-106	105-106	NA	diet	abdominal cavity	sarcoma	0/20 (p<0.001[c])
		M	200	105-106	105-106	99.5	diet	abdominal cavity	sarcoma	6/49
		M	400	105-106	105-106	99.5	diet	abdominal cavity	sarcoma	31/49 (p<0.001[d])
		F	0	105-106	105-106	NA	diet	multiple organs	fibrosarcoma	0/20
		F	200	105-106	105-106	99.5	diet	multiple organs	fibrosarcoma	0/50
		F	400	105-106	105-106	99.5	diet	multiple organs	fibrosarcoma	1/50
		M	0	105-106	105-106	NA	diet	multiple organs	fibrosarcoma	0/20 (p<0.001[c])
		M	200	105-106	105-106	99.5	diet	multiple organs	fibrosarcoma	0/49
		M	400	105-106	105-106	99.5	diet	multiple organs	fibrosarcoma	13/50 (p=0.007[d])
		F	0	105-106	105-106	NA	diet	multiple organs	osteosarcoma	0/20
		F	200	105-106	105-106	99.5	diet	multiple organs	osteosarcoma	0/50
		F	400	105-106	105-106	99.5	diet	multiple organs	osteosarcoma	3/50
		M	0	105-106	105-106	NA	diet	multiple organs	osteosarcoma	0/20

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TABLE 5-2 (cont.)

Exposure Route	Species/ Strain	Sex	Dose (ppm)	Duration of Treatment (weeks)	Duration of Study (weeks)	Purity of Compound (%)	Vehicle or Physical State	Target Organ	Tumor Type	Tumor Incidence (p value)[b]
Oral (cont.)		M	200	105-106	105-106	99.5	diet	multiple organs	osetosarcoma	0/49
		M	400	105-106	105-106	99.5	diet	multiple organs	osteosarcoma	3/50
Oral	mice/B6C3F1	F	0	105-106	105-106	NA	The incidence distribution and nature of tumors observed in dosed mice of both sexes were not significantly different from the controls.			
			208[e]			99.5				
			545[e]			99.5				
		M	0	105-106	105-106	NA	The incidence distribution and nature of tumors observed in dosed mice of both sexes were not significantly different from the controls.			
			200			99.5				
			400			99.5				

QUALITY OF EVIDENCE

Strengths of Study: Both sexes of rats and mice were treated for a significant portion of their lifespan. An adequate number of animals per species and sex were studied (50). Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. Two dosage levels were studied.

Overall Adequacy: Adequate

Comments: Under conditions of this bioassay, azobenzene was found to be carcinogenic for F344, inducing various types of sarcomas in the spleen and abdominal organs of males and females. Azobenzene was not carcinogenic for B6C3F1 mice.

[a]Source: NCI, 1979

[b]The p value is given for Cochran-Armitage test and Fisher Exact test as indicated by notes c and d. Where no p value is given, the linear trend or difference from control is not significant.

[c]Cochran-Armitage test for linear trend. The test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance.

[d]The one-tailed Fisher exact test was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level (0.05 level of significance).

[e]Time weighted average dose. Female mice were given 400 and 800 ppm azobenzene for 38 weeks and then changed to doses of 100 and 400 ppm, respectively, for 67 or 68 weeks.

NA - Not applicable

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In mice, no significant increased incidences of tumors occurred in dosed males or females compared with controls. It was concluded that under the conditions of the bioassay, no compound related changes were observed in B6C3F1 mice. The results of the NCI (1979) bioassay do not support the observed increased incidence of liver tumors in B6C3F1 mice in the study by Innes (1968) and Innes et al. (1969).

Chronic feeding of azobenzene to F344 rats was associated with increased incidences of malignant mesenchymal tumors not observed in control animals (see Table 5-2) (NCI, 1979). These tumors spread throughout the abdominal cavity involving multiple organs and tissues, such that determination of the primary site was often not possible. The spleen was most consistently affected, and the most frequently observed tumors were fibrosarcomas. Increased incidences of osteosarcomas and hemangiosarcomas were also observed in the spleen. Many fibrosarcomas and osteosarcomas involved multiple organs and these were considered to be extensions of the primary tumors of the spleen. The incidence of fibrosarcomas at multiple sites was significantly increased in high-dose males compared with controls ($p=0.007$). Highly significant ($p<0.001$) dose-related increased incidences of all sarcomas of the abdominal cavity were observed for both male and female rats. Based on the histopathologic examination, it was concluded that azobenzene was carcinogenic for male and female F344 rats (NCI, 1979; Weisburger, 1983; Goodman et al., 1984).

5.2. MUTAGENICITY

The results of mutagenicity testing of azobenzene are summarized in Table 5-3.

TABLE 5-3

Mutagenicity Testing of Azobenzene

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	Salmonella typhimurium TA100	purest commercial grade available	plate incorporation	10-1000 µg/plate	S9	+	379 revertant colonies per 50 µg tested - 1.4 revertants per nmol (data are from the linear region of the dose-response curve).	McCann et al., 1975
Reverse mutation	S. typhimurium TA 98 TA100 TA1535 TA1537	99% pure	preincubation, plate incorporation	3.3, 10, 33.3, 100 and 333.3 µg/plate	±S9	- ± - -	Positive response in the presence of S9 in TA100	Haworth et al., 1983
Reverse mutation	Escherichia coli Sd-4-73	NR	spot test	crystals	none	-	NC	Szybalski, 1958
Rec assay	Bacillus subtilis H17/M45 HL13g/HJ-15	purest commercial grade available	spot test, plate incorporation	NR	S9	no test result	No growth inhibition was observed in the wild type and the corresponding repair deficient mutants of all strains.	Suter and Jaeger, 1982
Rec assay	E. coli AB1157 compared with JC5547, JC2921, JC2926 and JC5519	purest commercial grade available	spot test, plate incorporation	NR	S9	no test result	No growth inhibition was observed in the wild type and the corresponding repair deficient mutants of all strains	Suter and Jaeger, 1982
Chromosomal aberration, lethal mutations	Drosophila melanogaster	NR	vapor (aerosol)	NR	NA	-	Control rate and number of sperm treated not reported; hence criteria for positive response uncertain	Demerec, 1948; IARC, 1975
Cytotoxicity and DNA damag	rat hepatocytes	purest commercial grade available	alkaline elution	0.03, 0.3 and 3.0 mM	NA	+	A 3-fold increase in elution rate compared with the control was considered an indication of a biologically significant increase in DNA damage: at 0.3 mM, the elution rate = 3.1- to 5.0-fold the control; at 3.0 mM, the elution rate >7.0-fold the control. Category 1-DNA single-strand breaks with <30% cytotoxicity	Sina et al., 1983

TABLE 5-3 (cont.)

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
UDS	UV-irradiated human lymphocytes	NR	cell culture	$4x10^{-5}$ M	none	+	The concentration of $4x10^{-5}$ M produced 50% inhibition of repair replication	Gaudin et al., 1971
Cell transformation	mammalian cells (BHK 21/C13)	appeared to be pure by the analytical techniques used	cell culture	0-100 µg/ml	S9	-	NC	Ashby et al., 1978

NR = Not reported; NC = no comment; NA = not applicable

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Azobenzene was positive for reverse mutation in the presence of S9 metabolic activation when tested by plate incorporation in Salmonella typhimurium strain TA100 (McCann et al., 1975; Haworth et al., 1983). Negative results were reported for reverse mutation by spot test in Escherichia coli (Szybalski, 1958) and for chromosomal aberrations in Drosophila melanogaster (Demerec, 1948). No growth inhibition was observed in wild type or repair deficient strains of Bacillus subtilis and E. coli in the rec assay (Suter and Jaeger, 1982). Azobenzene was positive for causing DNA single strand breaks with <30% cytotoxicity by the alkaline elution/rat hepatocyte assay (Sina et al., 1983). Positive results for UDS were obtained by Gaudin et al. (1971) with a concentration of $4x10^{-3}$ M azobenzene, resulting in 50% inhibition of repair replication in irradiated human lymphocytes. Azobenzene was found to be negative in the cell transformation assay (Ashby et al., 1978), having <50 transformants per 10^6 survivors.

5.3. TERATOGENICITY

Pertinent data regarding the teratogenicity of azobenzene could not be located in the available literature as cited in the Appendix.

5.4. OTHER REPRODUCTIVE EFFECTS

Pertinent data regarding other reproductive effects of azobenzene could not be located in the available literature as cited in the Appendix.

5.5. CHRONIC AND SUBCHRONIC TOXICITY

In a subchronic feeding study conducted to estimate the MTDs of azobenzene for administration in the NCI (1979) carcinogenic bioassay, groups of five male and five female F344 rats and B6C3F1 mice were administered azobenzene in the diet at 500-4600 ppm for 7 weeks, followed by a 1-week observation period (Table 5-4). Additional groups of five animals of each sex and species served as controls. Rats treated at ≥700 ppm had enlarged livers. Treatment-related histopathologic lesions were observed in the

TABLE 5-4

Azobenzene Subchronic Feeding Studies in Rats and Mice[a,b]

Dose (ppm)	Male			Female		
	Survival[c]	Week on Study When Last Death Occurred	Mean Weight at Week 7 as % of Control	Survival[b]	Week on Study When Last Death Occurred	Mean Weight at Week 7 as % of Control
Rats						
500	5/5	NA	88	5/5	NA	96
700	5/5	NA	75	5/5	NA	83
1000	5/5	NA	73	5/5	NA	69
2200	5/5	NA	33	0/5	6	NA
4600	0/5	2	NA	0/5	2	NA
Mice						
500	5/5	NA	88	5/5	NA	91
700	5/5	NA	86	5/5	NA	91
1000	5/5	NA	91	5/5	NA	91
2200	4/5	5	89	5/5	NA	91
4600	5/5	NA	66	5/5	NA	6/

[a] Source: NCI, 1979

[b] The animals were treated with azobenzene in the diet for 7 weeks, followed by a 1-week observation period.

[c] Number surviving/number in group

NA = Not applicable

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livers and kidneys of rats at 1000 or 2200 ppm. Mice treated at ≥700 ppm had enlarged spleens and mesenteric nodes. All mice examined had slight stasis of the bile, slight granular intracytoplasmic pigmentation of the proximal convoluted tubular epithelium and slight pigmentation of the splenic red pulp. It was not clear if these lesions were also observed in control mice.

In the chronic feeding study, groups of 50 male and 50 female F344 rats and 50 male and 50 female B6C3F1 mice were treated with azobenzene in the diet for 106 weeks (NCI, 1979). The low and high dietary levels were set at 200 and 400 ppm for male and female rats. For male mice, these levels were 200 and 400 ppm, and for female mice, 400 and 800 ppm; however, because of excessive decrease in body weight gain, doses for the low- and high-dose female mice were reduced to 100 and 400 ppm, respectively, after 38 weeks.

Dose-related decreased mean body weight of treated male and female rats were observed throughout the study. Survival until week 90 among male rats was 35/50 (70%) for the high-dose group, 47/50 (94%) for the low-dose group and 17/20 (85%) for the control group and among female rats was 48/50 (96%) of the high-dose group, 44/50 (88%) of the low-dose group and 19/20 (95%) of the control group. The Tarone test for positive dose-related trends in mortality was not significant in female rats but was significant in males ($p<0.001$). A departure from the linear trend was observed ($p<0.001$) because the low-dose animals survived longer than the controls.

Treatment-related nonneoplastic lesions were observed in both male and female rats. Incidences of nonproliferative lesions appeared at both dose levels and were not increased in the high-dose group. Hemosiderosis was observed in the liver, spleen and renal tubular epithelium of treated female rats. The effect was more pronounced in the high-dose group. Chronic capsulitis of the spleen, characterized by cystic papillary proliferation of

serosal cells, thickening of the capsule and focal collections of mononuclear cells and mineral deposits, was observed in all dose groups but was more evident in treated females. Unusual accumulations of mature appearing adipose tissue in the spleen were observed in low-dose rats of both sexes. Fibrosis of the splenic pulp was observed with varying degrees of severity in treated female rats.

Dose-related decreased mean body weights of female mice were observed throughout the study. The body weights of treated males were also lower than control, but a dose-response was not apparent. Survival to at least 90 weeks was 46/50 (92%) for the high-dose group, 49/50 (98%) for the low-dose group, and 18/20 (90%) for the control group for males and 37/50 (74%) for the high-dose group, 43/50 (86%) for the low-dose group and 19/20 (95%) for the control group for females. The Tarone test for positive dose-related trends in mortality was significant in females ($p<0.001$) but not males. No increased incidences of nonneoplastic lesions were observed in treated mice compared with controls.

As reviewed by U.S. EPA (1981), Scherf (1976) found that azobenzene was cytotoxic to antibody-forming cells of the spleen, resulting in altered cellular immune reactions in male Sprague-Dawley rats treated orally with 9.2 mg/kg/day for 20-60 days. Additional details were not provided.

5.6. OTHER RELEVANT INFORMATION

The oral lethal dose of azobenzene in humans was reported as 50 mg/kg (Weiss, 1980) and 50-500 mg/kg (Gosselin et al., 1976). According to Windholz (1976) and Sax (1984), the oral LD_{50} for rats is 1000 mg/kg.

Pretreatment of female Sprague-Dawley rats with 20-100 mg azobenzene resulted in weak induction of DMBA metabolism. Azobenzene also blocked DMBA-induced adrenal damage but only at high dosages (Levin and Conney, 1967; Conney and Levin, 1966).

6. AQUATIC TOXICITY

Data regarding the toxic effects of azobenzene to aquatic organisms are limited. Tonogai et al. (1982) investigated the acute toxicity of azobenzene to himedaka, Oryzias latipes, according to the procedures of the Japan Industrial Standards. The 24- and 48-hour TLm values were reported as 1.0 and 0.5 mg, respectively. Additional data on the acute and chronic toxicity and residual analysis of azobenzene on aquatic organisms could not be located in the available literature as cited in the Appendix.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

Pertinent guidelines and standards, including EPA ambient water and air quality criteria, drinking water standards, FAO/WHO ADIs, EPA or FDA tolerances for raw agricultural commodities or foods, and ACGIH, NIOSH or OSHA occupational exposure limits could not be located in the available literature as cited in the Appendix.

7.2. AQUATIC

Guidelines and standards for the protection of aquatic organisms from the toxic effects of azobenzene could not be located in the available literature as cited in the Appendix.

8. RISK ASSESSMENT

Although a greater incidence of hepatomas occurred in male B6C3F1 mice when compared with the controls, azobenzene was reported to be noncarcinogenic in male and female B6AKF1 and B6C3F1 mice when given 21.5 mg/kg by gavage for 7-28 days and then given 56 ppm in the diet for 78 weeks (Innes, 1968; Innes et al., 1969) (see Table 5-1). In another study (NCI, 1979), azobenzene was given in the diet to male B6C3F1 mice at concentrations of 0, 200 and 400 ppm and female B6C3F1 mice at TWA concentrations of 0, 208 and 545 ppm for 105-106 weeks. Results of the NCI (1979) bioassay (see Table 5-2) indicated that azobenzene was not carcinogenic in male or female B6C3F1 mice under conditions of the study. In the NCI (1979) study, male and female F344 rats were also administered azobenzene in the diet at 0, 200 and 400 ppm for 105-106 weeks. Azobenzene was found to be carcinogenic for F344 rats, inducing various types of sarcomas in the spleen and abdominal organs of both males and females. These tumors were so highly invasive that determining the primary tumor site was often precluded. The sites, types and incidences of these tumors are presented in Table 5-2.

Azobenzene was reported to be mutagenic in Salmonella typhimurium strain TA100 in the presence, but not in the absence of S9 (McCann et al., 1975; Haworth et al., 1983) and nonmutagenic in Escherichia coli (Szybalski, 1958). Negative results were reported for chromosomal aberrations in Drosophila melanogaster (Demerec, 1948) and in the cell transformation assay (Ashby et al., 1978). Azobenzene was positive for causing DNA single strand breaks with <30% cytotoxicity by the alkaline elution/rat hepatocyte assay (Sina et al., 1983) and for UDS in human lymphocytes (Gaudin et al., 1971). Results of mutagenicity testing of azobenzene were summarized in Table 5-3.

Pertinent data regarding teratogenic and reproductive effects of azobenzene could not be located in the available literature as cited in the Appendix.

Dietary administration of azobenzene to rats for 7 weeks resulted in deaths of 5/5 female rats at 2200 and 4600 ppm and 5/5 males at 4600 ppm (NCI, 1979). No deaths occurred at $\leq$1000 ppm; however, there was a dose-related decrease in mean body weight at $\geq$500 ppm. Enlarged livers and histopathologic changes in livers and kidneys were observed in rats treated at $\geq$700 ppm. No apparent effect on survival was noted in mice similarly treated, but body weights were substantially decreased at 4600 ppm. Enlarged spleens and mesenteric nodes were observed in mice at $\geq$700 ppm.

Chronic dietary exposure to azobenzene resulted in decreased mean body weights of male mice at 200 and 400 ppm and female mice at TWA levels of 208 and 545 ppm. For female mice, this effect was dose-related throughout the assay (NCI, 1979). A positive dose-related trend in mortality was significant in female mice ($p<0.001$) but not male mice.

Chronic dietary exposure to azobenzene resulted in dose-related decreased mean body weights of male and female F344 rats at 200 and 400 ppm (NCI, 1979). A positive dose-related trend in mortality was significant in male rats ($p<0.001$) but not in females. A departure from the linear trend ($p<0.001$) was also observed since the low-dose male rats survived longer than the controls. Nonproliferative lesions associated with chronic azobenzene administration were observed in treated male and female rats. Hemosiderosis was observed in the livers, spleen and renal tubular epithelium of treated female rats. Chronic capsulitis of the spleen was observed

in all dose groups but was more evident in the females. Unusual accumulations of mature-appearing adipose tissue in the spleen of low-dose males and females and varying degrees of fibrosis of the splenic pulp in low-dose females were also observed.

Based on the histopathologic findings of the long-term feeding study of azobenzene (NCI, 1979), it was concluded that azobenzene was carcinogenic for F344 rats, inducing various types of sarcomas in the spleen and other abdominal organs of male and female rats. The dose-related trend and the increased incidence of abdominal cavity sarcomas in high-dose rats compared with controls were both highly significant ($p<0.001$). Therefore, it is appropriate to derive a q_1^* for humans for azobenzene based on the dose-response data for abdominal cavity sarcomas. The tumor incidences and data used to calculate the q_1^* for azobenzene are presented in Tables 8-1 and 8-2. The unadjusted q_1^* was calculated using the computerized multistage linear model developed by Kenneth Crump and adopted by the U.S. EPA (Federal Register, 1980). The factor used to convert the adjusted q_1^* from animal data to a q_1^* for humans (assuming 70 kg bw) was the cube root of the ratio of the body weight of humans to the body weight of rats. The animal body weights used were taken from data presented in the NCI (1979) report (female rats = 0.25 kg; male rats = 0.375 kg). Experimental doses of 0, 200 and 400 ppm were transformed to 0, 10 and 20 mg/kg/day, respectively, using standard food consumption estimates for rats (f = 0.05 kg food/kg bw/day).

The q_1^* value for humans obtained using the data for female rats is 1.08×10^{-1} $(mg/kg/day)^{-1}$ and using the data for male rats is 7.31×10^{-2} $(mg/kg/day)^{-1}$. Since the q_1^* of 1.08×10^{-1} $(mg/kg/day)^{-1}$ is higher, it is recommended for estimating the increased lifetime cancer risk for exposure of humans to azobenzene.

TABLE 8-1

Derivation of q_1^* from Female Rat Data

Compound: Azobenzene

Reference: NCI, 1979

Species, Strain, Sex: Rat, F344, female

Body weight: 0.25 kg (measured)

Length of exposure (le) = 106 weeks

Length of experiment (Le) = 106 weeks

Lifespan of animal (L) = 106 weeks

Tumor site and type: abdominal cavity, sarcoma

Route, vehicle: oral, diet

Experimental Doses or Exposures (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested or Examined
0	0	0/20
200	10	5/50
400	20	21/50

Unadjusted q_1^* from study = 1.6456571×10^{-2} $(mg/kg/day)^{-1}$
Human q_1^* = 1.0766107×10^{-1} $(mg/kg/day)^{-1}$

TABLE 8-2

Derivation of q_1^* from Male Rat Data

Compound: Azobenzene

Reference: NCI, 1979

Species, Strain, Sex: Rat, F344, male

Body weight: 0.375 kg (measured)

Length of exposure (le) = 106 weeks

Length of experiment (Le) = 106 weeks

Lifespan of animal (L) = 106 weeks

Tumor site and type: abdominal cavity, sarcoma

Route, vehicle: oral, diet

Experimental Doses or Exposures (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested or Examined
0	0	0/20
200	10	6/49
400	20	31/49

Unadjusted q_1^* from study = 1.2797641×10^{-2} $(mg/kg/day)^{-1}$
Human q_1^* = 7.3139534×10^{-2} $(mg/kg/day)^{-1}$

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

Two chronic carcinogenicity studies of azobenzene in animals were available (Innes, 1968; NCI, 1979); however, only the NCI (1979) bioassay provided information on toxic effects other than carcinogenicity. This study was discussed in Section 5.5. and is summarized in Table 9-1. In addition to the effects presented in Table 9-1, chronic dietary exposure to azobenzene resulted in significant dose-related increased incidences of sarcomas of the abdominal cavity of rats; the incidences were significantly greater than control in the high-dose males and females. No increased incidences of tumors were observed in mice.

As indicated in Table 9-1, there was a dose-related decrease in mean body weight gain in male and female rats and increased mortality in high-dose males. The chronic capsulitis of the spleen observed in all treated rat groups was described as "cystic papillary proliferation of serosal cells, thickening of the capsule, and focal collections of mononuclear cells and mineral deposits" (NCI, 1979). Female rats at both doses had hemosiderosis of the spleen, liver and kidney.

Decreased body weight gains also occurred in all treated groups of mice. In female mice, the decrease was dose-related throughout the study, leading to a reduction of the dietary levels from 400 and 800 ppm to 100 and 400 ppm, respectively, after 38 weeks resulting in TWA levels of 208 and 545 ppm or 27 and 71 mg/kg/day. Female mice also had dose-related increased mortality at 27 and 71 mg/kg/day; survival at 90 weeks was 74% for the high-dose group, 86% for the low-dose group and 95% for controls. Although it cannot be determined based on the available data if a lower dose would also result in increased mortality, the 27 mg/kg/day dose appears to be near the threshold for this effect in female mice. The dietary levels in the chronic study

TABLE 9-1

Oral Toxicity Summary for Azobenzene[a]

Species/ Strain	Sex	No. at Start	Vehicle	Dosage/ Exposure	Dose[b] (mg/kg/day)	Response
Rats/F344	M	50	diet	200 ppm 106 weeks	10	Decreased body weight (dose-related), chronic splenic capsulitis, accumulation of adipose tissue in spleen
	F	50	diet	200 ppm 106 weeks	10	Decreased body weight (dose-related), chronic splenic capsulitis, hemosiderin deposited in liver spleen and renal tubular epithelium, accumulation of adipose tissue in spleen
	M	50	diet	400 ppm 106 weeks	20	Dose-related decrease in body weight, increase in mortality, chronic capsulitis of spleen
	F	50	diet	400 ppm 106 weeks	20	Dose-related decrease in body weight, hemosiderosis of spleen, liver and renal tubular epithelium
Mice/B6C3F1	M	50	diet	200 ppm 106 weeks	26	Decreased body weight
	F	50	diet	208 ppm[c] 106 weeks	27	Decreased body weight (dose-related), increased mortality (dose-related)

TABLE 9-1 (cont.)

Species/ Strain	Sex	No. at Start	Vehicle	Dosage/ Exposure	Dose[b] (mg/kg/day)	Response
Mice/B6C3F1 (cont.)	M	50	diet	400 ppm 106 weeks	52	Decreased body weight
	F	50	diet	545 ppm[c] 106 weeks	71	Dose-related decrease in body weight, dose-related increase in mortality (37/50 survived)

[a]Source: NCI, 1979

[b]Dose calculated from dietary level assuming that a rat and a mouse consumes a daily amount of food equal to 5 and 13%, respectively, of their body weight.

[c]THA concentration

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were chosen based on the results of a 7-week feeding study in which no female mice treated at up to 4600 ppm (598 mg/kg/day) died (NCI, 1979). Furthermore, no increased mortality was observed in male mice at 200 or 400 ppm (26 or 52 mg/kg/day) in the chronic study. Therefore, the 27 mg/kg/day dose level can be considered an MED for increased mortality in female mice.

The data used to calculate CSs and RQs are presented in Table 9-2. The rat dosages were converted to human MEDs of 107.0 and 245.0 mg/kg/day, respectively, by multiplying the cube root of the ratio of the rat weight to the weight of a man (assumed to be 70 kg), and by 70 kg to adjust to units of mg/day. Rat body weights of 0.25 and 0.375 kg reported by NCI (1979) were used. The chronic dosages of 26 and 27 mg/kg/day for mice were also converted to human MEDs. A body weight for mice of 0.04 kg (NCI, 1979) was used for the body weight ratio approximation. The MED of 107.0 mg/day obtained from the data for rats (see Table 9-2) corresponds to an RV_d of 2.5. The effects of decreased body weight, capsulitis of the spleen and hemosiderin in multiple organs (see Tables 9-1 and 9-2) were assigned an RV_e of 6. Multiplying the RV_d by the RV_e results in a CS of 15, which corresponds to an RQ of 1000. The remaining values reported in Table 9-2 were determined as described above. The highest CS, 22, which corresponds to an RQ of 100 based on increased mortality in mice (NCI, 1979), was used for the ranking of azobenzene (Table 9-3).

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

The available information regarding the carcinogenicity of azobenzene was discussed in Section 5.1. and summarized in Tables 5-1 and 5-2. Chronic dietary exposure to azobenzene or a single subcutaneous injection of azobenzene did not result in significantly increased incidences of tumors compared with controls in B6C3F1 or B6AKF1 mice (Innes, 1968; Innes et al.,

TABLE 9-2

Composite Scores for Oral Toxicity of Azobenzene[a]

Species/Strain	Dose/Exposure	Human MED (mg/day)	RV_d	Effect	RV_e	CS	RQ
Rat/F344	10 mg/kg/day 106 weeks	107.0[b]	2.5	Body weight decrease, chronic splenic capsulitis, hemosiderin deposited in liver, spleen and renal tubular epithelium	6	15	1000
Rat/F344	20 mg/kg/day 106 weeks	245.0[c]	1.9	Increased mortality	10	19	1000
Mouse/B6C3F1	26 mg/kg/day 106 weeks	151.0[d]	2.2	Decreased body weight gain	4	8.8	1000
Mouse/B6C3F1	27 mg/kg/day[e] 106 weeks	156.8[d]	2.2	Increased mortality	10	22	100

[a]Source: NCI, 1979

[b]Body weights for female rats = 0.25 kg

[c]Body weights for male rats = 0.375 kg

[d]Body weight for mice = 0.04 kg

[e]TWA dose

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TABLE 9-3

Azobenzene

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral/diet
Dose*:	156.8 mg/day
Effect:	increased mortality
Reference:	NCI, 1979
RV_d:	2.2
RV_e:	10
Composite Score:	22
RQ:	100

*Equivalent human dose

1969; NCI, 1979). Sherman rats treated with weekly subcutaneous injections of azobenzene also failed to develop tumors (Spitz et al., 1950; data not included in Table 5-1 or 5-2). Chronic dietary exposure of F344 rats to azobenzene, however, resulted in significant dose-related increased incidences of sarcomas of the abdominal cavity, primarily in the spleen, of males and females (see Table 5-2). The incidences in the high-dose groups were significantly greater than in the control groups. Therefore, there is sufficient evidence that azobenzene is an animal carcinogen. The Clearinghouse on Environmental Carcinogens (NCI, 1979) concluded that the data in rats indicated that azobenzene may be a potential carcinogen for humans, although there is no direct evidence of an association between exposure and human cancer. Additional evidence for carcinogenicity of azobenzene includes an increased incidence of hepatomas in male mice and positive in vitro tests for mutagenicity. It should also be noted that azobenzene may be nonenzymatically converted to the known human carcinogen benzidene under the acidic conditions normally encountered in the stomach. Also, following its absorption into the body, azobenzene may be metabolized to benzidene. Using the U.S. EPA Proposed Guidelines for Carcinogen Risk Assessment, azobenzene is classified as having sufficient animal evidence and inadequate epidemiologic evidence. The overall weight of evidence classification is B-2 meaning that azobenzene is probably carcinogenic in humans.

The dose-response data in female and male F344 rats for increased incidences of abdominal cavity sarcomas were used to calculate potency factors (unadjusted $1/ED_{10}$) for azobenzene (Table 9-4) using the linearized multistage model developed by Crump and adopted by the U.S. EPA (Federal Register, 1980). The resultant values for the unadjusted $1/ED_{10}$ were converted to F factors for humans by multiplying by the cube root of the

ratio of the weight of a man (assumed to be 70 kg) by the weight of the rat (0.25 kg and 0.375 kg for females and males, respectively, as reported in the study). The F factor of 7.23×10^{-1} $(mg/kg/day)^{-1}$ obtained using data for female rats is the higher value. Since the F factor is <1, azobenzene is placed in Potency Group 4 and, therefore, ranks low in the Hazard Ranking Scheme for Reportable Quantities under CERCLA.

TABLE 9-4

Derivation of Potency Factor (F)
Agent: Azobenzene

Reference:	NCI, 1979					
Exposure route:	oral					
Species:	rat					
Strain:	F344					
Sex:	female and male					
Vehicle or physical state:	diet					
Body weight: (reported)	0.25 kg for females; 0.375 kg for males					
Duration of treatment:	106 weeks					
Duration of study:	106 weeks					
Lifespan of animal:	106 weeks					
Target organ:	abdominal cavity					
Tumor type:	sarcoma					
	Females			Males		
Experimental doses/ exposure (ppm):	0	200	400	0	200	400
Transformed doses (mg/kg/day):	0	10	20	0	10	20
Tumor incidence:	0/20	5/50	21/50	0/20	6/49	31/50
Unadjusted $1/ED_{10}$:	1.1056745×10^{-1} $(mg/kg/day)^{-1}$			1.4328019×10^{-1} $(mg/kg/day)^{-1}$		
F factor:	7.2334693×10^{-1} $(mg/kg/day)^{-1}$			8.1885763×10^{-1} $(mg/kg/day)^{-1}$		

10. REFERENCES

Aftergut, S. 1979. Deformation recording media. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., Vol. 7. John Wiley and Sons, Inc., New York. p. 457.

Ali, A.M.J. and Z.Y. Al-Saigh. 1980. Photodecomposition of azobenzenes. J. Chem. Technol. Biotechnol. 30: 440-446.

Ashby, J., J.A. Styles and D. Paton. 1978. In vitro evaluation of some derivatives of the carcinogen Butter Yellow: Implications for environmental screening. Br. J. Cancer. 38(1): 34-50.

Baker, M.J., S.E. Buday, C.L. Gandenberger, K.L. Gandenberger, E.G. Giordano and J.B. Merz, Ed. 1978. Chem. Sources, U.S.A. Directories Publishing Company, Inc., Flemington, NJ. p. 80. (Cited in U.S. EPA, 1981)

Bordeleau, L.M. and R. Bartha. 1972. Biochemical transformations of herbicide-derived anilines requirements of molecular configuration. Can. J. Microbiol. 18: 1873-1882.

Bray, H.G., R.C. Clowes and W.V. Thorpe. 1951. The metabolism of azobenzene and p-hydroxyazobenzene in the rabbit. Biochem. J. 49: 115.

Briggs, G.G. 1981. Theoretical and experimental relationships between soil adsorption, octanol-water partition coefficient, water solubilities, bioconcentration factors and the parachor. J. Agric. Food Chem. 29: 1050-1059.

Conney, A.H. and W. Levin. 1966. Induction of hepatic 7,12-dimethylbenz-(α)anthracene metabolism by polycyclic aromatic hydrocarbons and aromatic azo derivatives. Life Sci. 5: 465-471.

Demerec, M. 1948. Mutations induced by carcinogens. Br. J. Cancer. 2: 114-117.

Elson, L.A. and F.L. Warren. 1944. The metabolism of azo compounds. 1. Azobenzene. Biochem. J. 38: 217-220.

Federal Register. 1980. Guidelines and Methodology Used in the Preparation of Health Effects Assessment Chapters of the Consent Decree Water Criteria Documents. 45 FR 79347-79357.

Fouts, J.R., J.J. Kamm and B.B. Brodie. 1957. Enzymatic reduction of prontosil and other azo dyes. J. Pharmacol. Exp. Ther. 120: 291-300.

Gaudin, D., R.S. Gregg and K.L. Yielding. 1971. DNA repair inhibition. Possible mechanism of action of cocarcinogens. Biochem. Biophys. Res. Commun. 45(3): 630-636.

Goodman, D.G., J.M. Ward and W.D. Reichardt. 1984. Splenic fibrosis and sarcomas in F344 rats fed diets containing aniline hydrochloride, p-chloro-aniline, azobenzene, o-toluidine hydrochloride, 4-4'-sulfonyldianiline, or D&C Red No. 9. J. Natl. Cancer Inst. 73(1): 265-273.

Gosselin, R.E., H.C. Hodge, R.P. Smith and M.N. Gleason. 1976. Clinical Toxicology of Commercial Products, 4th ed. The Williams and Wilkins Co., Baltimore, MD. p. 188. (Cited in U.S. EPA, 1981)

Griffiths, J. 1972. Photochemistry. II. Photochemistry of azobenzene and its derivatives. Chem. Soc. Rev. 1(4): 481-493.

Hansch, C. and A.J. Leo. 1981. Medchem project. Issue No. 19. Pomona College, Claremont, CA.

Hawley, G.G. 1977. The Condensed Chemical Dictionary, 9th ed. Van Nostrand Reinhold Co., New York, NY. p. 83.

Haworth, S., T. Lawlor, K. Mortelmans, W. Speck and E. Ziegler. 1983. Salmonella mutagenicity tests for 250 chemicals. Environ. Mutagen. Suppl. 1: 3-142.

Hoefnagel, M.A., A. Van Veen and B.M. Webster. 1969. Protonation of azo-compounds. Part II. The structure of the conjugate acid of trans-azoben-zene. Rec. Trav. Chim. 88: 562.

IARC (International Agency for Research on Cancer). 1975. Azobenzene. In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man: Some Aromatic Azo Compounds. WHO, IARC, Lyons, France. 8: 75-81.

Innes, J.R.M. 1968. Evaluation of carcinogenic, teratogenic, and mutagenic activities of selected pesticides and industrial chemicals. Vol. 1, Carcinogenic study. Bionetics Research Labs, Inc., Bethesda National Technical Information Service, U.S. Department of Commerce.

Innes, J.R.M., B.M. Ulland, M.G. Valerio, et al. 1969. Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. J. Natl. Cancer Inst. 43: 1101-1114.

Jungclaus, G.A., V. Lopez-Avila and R.A. Hites. 1978. Organic compounds in an industrial waste water: A case study of their environmental impact. Environ. Sci. Technol. 12: 88-96.

Kerzhner, B.K., P.N. Taran and M.A. Shevchenko. 1980. Oxidation of azobenzene with ozone in dilute aqueous solutions. Khim. Tekhnol. Vody. 2(2): 127-129. (Rus.) (CA 94:52256y)

Levin, W. and A.H. Conney. 1967. Stimulatory effect of polycyclic hydrocarbons and aromatic azo derivatives on the metabolism of 7,12-dimethylbenz-[a]anthracene. Cancer Res. 27(11): 1931-1938.

Lyman, W.J., W.F. Reehl and D.H. Rosenblatt. 1982. Handbook of Chemical Property Estimation Methods. Environmental Behavior of Organic Compounds. McGraw-Hill, Inc., New York, NY. p. 5-1 to 5-30.

Malaney, G.W. 1960. Oxidative abilities of aniline-acclimated activated sludge. J. Water Pollut. Control Fed. 32: 1300.

McCann, J., E. Choi, E. Yamasaki and B.N. Ames. 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Natl. Acad. Sci. USA 72(12): 5135-5139.

Meir, H. 1971. The photochemistry of dyes. In: The Chemistry of Synthetic Dyes, Vol. IV, K. Venkataraman, Ed. Academic Press, NY. p. 389. (Cited in Radding et al., 1977; U.S. EPA, 1981)

NCI (National Cancer Institute). 1979. Bioassay of azobenzene for possible carcinogenicity. NCI Carcinogenesis Technical Report Series. No. 154. 112 p. U.S. DHEW Publ. No. (NIH) 79-1710.

Northcott, J. 1978. Amines, aromatic (aniline). In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., Vol. 2. John Wiley and Sons, Inc., New York, NY. p. 312-321.

Pillai, P., C.S. Helling and J. Dragun. 1982. Soil-catalyzed oxidation of aniline. Chemosphere. 11: 299-317.

Radding, S.B., D.H. Liu, H.L. Johnson and T. Mill. 1977. Review of the environmental fate of selected chemicals. EPA 560/5-77-003. p. 83-86. (Cited in U.S. EPA, 1981)

Sadtler. 1966. Sadtler Standard Ultraviolet Spectra. Sadtler Research Laboratories, Inc., Philadelphia, PA. Spectrum 136.

Sandridge, R.L. and H.S. Staley. 1978. Amines by reduction. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., Vol. 2. John Wiley and Sons, Inc., New York. p. 355-376.

Sax, N.I. 1984. Dangerous Properties of Industrial Materials, 6th ed. Van Nostrand Reinhold Company, New York, NY. p. 339.

Scherf, H.R. 1976. The effect of 4-dimethylaminobenzene and azobenzene on the immune reaction of male Sprague-Dawley rats. Z. Krebforsch. 8(7): 327-332. (Cited in U.S. EPA, 1981)

Shackelford, W.M. and L.H. Keith. 1976. Frequency of organic compounds identified in water. U.S. GPO, Washington, DC. EPA-600/4-76-062. p. 62. (Cited in U.S. EPA, 1981)

Sina, J.F., C.L. Bean, G.R. Dysart, V.I. Taylor and M.O. Bradley. 1983. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat. Res. 113(5): 357-391.

Spitz, S., W.H. Maguigan and K. Dobriner. 1950. The carcinogenic action of benzidine. Cancer. 3: 789-804.

Suter, W. and I. Jaeger. 1982. Comparative evaluation of different pairs of DNA repair-deficient and DNA repair-proficient bacterial tester strains for rapid detection of chemical mutagens and carcinogens. Mutat. Res. 97(1): 1-18.

Szybalski, W. 1958. Special microbiological systems. II. Observations on chemical mutagenesis in microorganisms. Ann. N.Y. Acad. Sci. 76: 475-489.

Takagishi, T., A. Katayama and N. Kuroki. 1969. Solubilities of azobenzene derivatives in water. Kolloid Z.Z. Polym. 232: 693-699.

Tonogai, Y., S. Ogawa, Y. Ito and M. Iwaida. 1982. Actual survey on TLm (median tolerance limit) values of environmental pollutants, especially on amines, nitriles, aromatic nitrogen compounds and artificial dyes. J. Toxicol. Sci. 7(3): 193-203.

Toxicology Data Bank. 1979. Azobenzene. (Computer Compilation of Data). Oak Ridge National Laboratory, Oak Ridge, TN. (Cited in U.S. EPA, 1981)

U.S. EPA. 1981. Chemical Hazard Information Profile. Draft Report. Azobenzene. U.S. EPA, Washington, DC.

U.S. EPA. 1985. TSCA (Toxic Substances Control Act). Plant and production search system. Search through CIS.

USITC (U.S. International Trade Commission). 1984. Imports of benzenoid chemicals and products, 1983. USITC Publ. 1548, Washington, DC. p. 10.

Weast, R.C., Ed. 1972. Handbook of Chemistry and Physics, 54th ed. Chemical Rubber Co., Cleveland, OH. p. C126.

Weisburger, E.K. 1983. Species differences in response to aromatic amines. Basic Life Sci. 24: 23-47.

Weiss, G., Ed. 1980. Hazardous Chemicals Data Book. Noyes Data Corp., Park Ridge, NJ. p. 963. (Cited in U.S. EPA, 1981)

Windholz, M., Ed. 1976. The Merck Index, 9th ed. Merck and Co., Rahway, NJ. p. 925.

Zoeteman, B.C.J., K. Harmsen, J.B.H.J. Linders, C.F.H. Morra and W. Slooff. 1980. Persistent organic pollutants in river water and groundwater of the Netherlands. Chemosphere. 9: 231-249.

APPENDIX

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

CA SEARCH (Files 308, 309, 310, 311, 320
TOXLINE
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP

These searches were conducted in February, 1985. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1980. Documentation of the Threshold Limit Values, 4th ed. (Includes Supplemental Documentation, 1981, 1982, 1983). Cincinnati, OH. 486 p.

ACGIH (American Conference of Governmental Industrial Hygienists). 1983. TLVs: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1983-1984. Cincinnati, OH. 94 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2A. John Wiley and Sons, NY. 2878 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2B. John Wiley and Sons, NY. p. 2879-3816.

Clayton, G.D. and F.E. Clayton, Ed. 1982. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2C. John Wiley and Sons, NY. p. 3817-5112.

Grayson, M. and D. Eckroth, Ed. 1978-1983. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. John Wiley and Sons, NY. 23 Volumes.

Hamilton, A. and H.L. Hardy. 1974. Industrial Toxicology, 3rd ed. Publishing Sciences Group, Inc., MA. 575 p.

IARC (International Agency for Research on Cancer). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. WHO, IARC, Lyons, France.

ITII (International Technology Information Institute). 1982. Toxic and Hazardous Industrial Chemicals Safety Manual for Handling and Disposal with Toxicity and Hazard Data. ITII, Tokyo, Japan. 700 p.

NTP (National Toxicology Program). 1984. Toxicology Research and Testing Program. Chemicals on Standard Protocol. Management Status.

Ouellette, R.P. and J.A. King. 1977. Chemical Week Pesticide Register. McGraw-Hill Book Co., NY.

Sax, I.N. 1979. Dangerous Properties of Industrial Materials, 5th ed. Van Nostrand Reinhold Co., NY.

SRI (Stanford Research Institute). 1984. Directory of Chemical Producers. Menlo Park, CA.

U.S. EPA. 1982. Chemical Activities Status Report, 3rd ed. (EPACASR). Offices of Pesticides and Toxic Substances, Washington, DC. EPA 560/TIIS-82-002b.

U.S. EPA. 1983. Status Report on Rebuttable Presumption Against Registration (RPAR) or Special Review Process. Registration Standards and the Data Call in Programs. Office of Pesticide Programs, Washington, DC.

U.S. EPA. 1984. CSB Existing Chemical Assessment Tracking System. Name and CAS Number Ordered Indexes. Office of Toxic Substances, Washington, DC.

USITC (U.S. International Trade Commission). 1983. Synthetic Organic Chemicals. U.S. Production and Sales, 1982, USITC Publ. 1422. Washington, DC.

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., NY.

Worthing, C.R. and S.B. Walker, Ed. 1983. The Pesticide Manual. British Crop Protection Council. 695 p.

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

Battelle's Columbus Laboratories. 1971. Water Quality Criteria Data Book. Volume 3. Effects of Chemicals on Aquatic Life. Selected Data from the Literature through 1968. Prepared for the U.S. EPA under contract No. 68-01-0007. Washington, DC.

Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. U.S. Dept. Interior, Fish and Wildlife Serv. Resource Publ. 137. Washington, DC.

McKee, J.E. and H.W. Wolf. 1963. Water Quality Criteria, 2nd ed. Prepared for the Resources Agency of California. State Water Quality Control Board. Publ. No. 3-A.

Pimental, D. 1971. Ecological Effects of Pesticides on Non-Target Species. PB-269605. Prepared for the U.S. EPA, Washington, DC.

Schneider, B.A. 1979. Toxicology Handbook. Mammalian and Aquatic Data. Book 1: Toxicology Data. U.S. EPA, Office Pesticide Programs, Washington, DC. EPA 540/9-79-003. NTIS PB 80-196876.